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*Historical Article—*

# The History of Infectious Bursal Disease: The Second Period Between 1977 and 2005

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SUMMARY. Major progress has been achieved since the first historical review of infectious bursal disease (H. N. Lasher and V. S. Davis, *Avian Diseases,* Vol. 41, pp. 11–19; 1997), much of it between 1977 and 2005. Significant findings in the 1980s were the presence of serotype 2 of infectious bursal disease virus (IBDV) and the diversity of antigenic and immunogenic types of IBDV. In the late 1980s, very virulent IBDV strains emerged and became widespread in many countries by the late 1990s. Soon after the discovery of the antigenic variants, specific commercial vaccines were developed and used successfully in the field. The structure of the virus was discovered, which led to the elucidation of virus genes being responsible for some of the virus' biological functions, including immunogenicity. A consequence of these findings was the development of a new class of recombinant vaccines, which were commercially licensed. Reverse genetics became another tool for virus characterization. The development of monoclonal antibodies allowed the identification of immunoglobulin M positive (IgM+) B cells as the major target cells for infection. A role of macrophages and T cells in IBDV pathogenesis and pathology of the bursa of Fabricius was suggested. New tools for serology and virus identification—ELISA and reverse transcriptase (RT) PCR, respectively—provided new insights in the epidemiology. The widespread use of ELISA kits facilitated the use of vaccines in the face of maternally derived antibodies against IBDV, allowing the determination of time of vaccine breakthrough and therefore vaccine administration.

RESUMEN. *Artículo histórico-* Historia de la enfermedad infecciosa de la bolsa: El segundo periodo entre 1977 y 2005.

Se han logrado importantes avances desde la primera revisión histórica de la enfermedad infecciosa de la bolsa (H. N. Lasher y V. S. Davis, Avian Diseases, vol. 41, págs. 11-19; 1997), gran parte entre los años 1977 y 2005. Hallazgos significativos en la década de 1980 fueron la detección de la presencia del serotipo 2 de la enfermedad infecciosa de la bolsa y la diversidad de tipos antigénicos e inmunogénicos del virus de la enfermedad infecciosa de la bolsa. A finales de la década de 1980, surgieron cepas muy virulentas de este virus, que se extendieron por muchos países a finales de la década de 1990. Poco después del descubrimiento de las variantes antigénicas, se desarrollaron vacunas comerciales específicas que se utilizaron con éxito en el campo. Se descubrió la estructura del virus, lo que condujo a la elucidación de los genes virales responsables de algunas de las funciones biológicas del virus, incluyendo la inmunogenicidad. Una consecuencia de estos hallazgos fue el desarrollo de una nueva clase de vacunas recombinantes, que obtuvieron licencias comerciales. La genética inversa se convirtió en otra herramienta para la caracterización del virus. El desarrollo de anticuerpos monoclonales permitió la identificación de células B IgM+ como las principales células blanco para la infección. Se sugirió un papel de los macrófagos y las células T en la patogénesis del virus de la enfermedad infecciosa de la bolsa y la patología de la bolsa de Fabricio. Nuevas herramientas para la serología y la identificación del virus como ELISA y PCR con transcriptasa inversa (RT), respectivamente, proporcionaron nuevos conocimientos en la epidemiología. El uso generalizado de estuches de ELISA facilitó el uso de vacunas frente a anticuerpos de origen materno contra el del virus de la enfermedad infecciosa de la bolsa, lo que permitió la determinación del momento en que la vacuna puede sobrepasar esos anticuerpos y por lo tanto, el momento para la administración de la vacuna.

Key words: infectious bursal disease, history, pathogenesis, variant strains of IBDV, vaccines

Abbreviations: BF = bursa of Fabricius; CAM = chorioallantoic membrane; CIAV = chicken infectious anemia virus; CMI = cell-mediated immunity; COST action 839 = European Cooperation in Science and Technology; CTL = cytotoxic T lymphocytes; dpi = days postinfection; $EID_{50}$ = embryo infectious $dose_{50}$; HG = Harderian gland; HVT = herpesvirus of turkeys; IBD(V) = infectious bursal disease (virus); IBV = infectious bronchitis virus; IFA = immunofluorescence antibody; MBL = Maine Biologics Laboratory; MDA = maternally derived antibodies; MD(V) = Marek's disease (virus); ND(V) = Newcastle disease (virus); NK = natural killer; NTF = National Turkey Federation (USA); OIE = Office International des Epizooties; PBL = peripheral blood lymphocytes; SPF = specific-pathogen-free; SRBC = sheep red blood cells; VN = virus-neutralizing; VP = virus protein; vv = very virulent; WOAH = World Organization for Animal Health (formerly OIE)

Infectious bursal disease (IBD) was first recognized by Cosgrove in 1957 and reported in 1962 (1). The disease was detected in the village of Gumboro, Delaware, and was referred to as Gumboro disease, with a possible viral origin. Initially, the agent was believed to be identical to the Gray strain of infectious bronchitis virus (IBV) (2), but Hitchner (3) noted clear differences between the new disease and infectious bronchitis. Soon afterwards, a virus clearly different from IBV was isolated by Winterfield (reviewed in Lasher and Davis [2]). The key to successful isolation was the use of embryonated eggs from chickens free of antibodies to this new pathogen and the use of the chorioallantoic membrane (CAM) route of inoculation (4). Soon after the isolation of the virus, the first nonattenuated vaccine came to use (5,6). Subsequently, attenuated vaccines became available, with Lasher and associates playing a major role in

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getting vaccines licensed (2). Another early key finding was the observation that infection with IBD virus (IBDV) caused severe immunosuppression. Cho referred to IBDV infection as biological bursectomy (7) when he noticed that infection of 1-day-old chicks with IBDV caused increased incidence of Marek's disease (MD) after challenge with MD virus (MDV). Staples (8) noted that several disease syndromes (e.g., gangrenous dermatitis, septicemia, etc.) were linked to clinically silent infections with IBDV. Rosenberger et al. (9) confirmed Staples' observations that progeny with these disease problems came mostly from IBDV-antibody–negative parent flocks. It is important to note that many of these diseases also are linked to infection with chicken infectious anemia virus (CIAV). CIAV was not isolated in the United States until 1988 (10,11,12), although CIAV antibodies were present in sera banked in 1959 (13). Thus, it is certainly feasible that some of the original studies described a combination of infection with IBDV and CIAV. For example, Schat et al. (14) reported on the pathogenesis of IBD in embryonally bursectomized chickens finding IBDV-positive cells in the thymus by direct immunofluorescence antibody (IFA) assay. However, it was learned in 1992 that the IBDV isolates used in this study were positive for CIAV (15). Because the antiserum for the IFA assay was prepared from sera from chickens infected with these isolates, it is likely that the positive cells in the thymus were actually CIAV-positive cells. However, others suggested the presence of IBD antigen in cells circulating through thymus tissue (16).

Lasher and Davis (2) described the early history of IBD covering approximately the first 20 yr after the first report by Cosgrove; in this paper, the history of events and discoveries related to the disease in the period from 1977 to around 2005 are described. During that time IBDV was detected in additional countries throughout the world, attracting many international research groups to work collaboratively on IBD. It is impossible to have an all-embracing list, as over 1000 manuscripts related to the topic IBDV were published between 1977 and 2005, with roughly 20 review articles in internationally peer-reviewed journals. Therefore, this history article will not cover all discoveries made worldwide during that time, but will focus on some of the highlights, especially related to work conducted in the United States and Europe; in other regions of the world research on IBDV was picked up with more emphasis during later years.

In Europe the COST action 839 (European Cooperation in Science and Technology, an interdisciplinary research network funded by the EU) was initiated (1999–2002) with about 100 scientists from around 20 countries in the EU and beyond, interested in immunosuppressive diseases in poultry, specifically also IBD. The different work packages targeted topics including epidemiology, diagnosis and economic impact, vaccination, and pathogenesis, as well as molecular virology. In 2001 Erhard F. Kaleta, head of the Institute of Poultry Diseases of the Justus Liebig University in Giessen, initiated the Second International Symposium of Infectious Bursal Disease and Chicken Infectious Anaemia in Rauischholzhausen (Germany, June 16–20, 2001) to bring together the international IBDV community as well as to communicate the efforts of the COST action in the international context. Nicolas Eterradossi from the World Organization for Animal Health (WOAH, former Office International des Epizooties [OIE]) Reference Laboratory for Infectious Bursal Disease (Ploufragan, France), summarized the most important new findings between 1994 and 2001, since the First International Symposium of Infectious Bursal Disease and Chicken Infectious Anaemia at the same place (17).

In the following we will summarize important key findings, including the discovery of IBDV variants and strains of high virulence (very virulent [vv] IBDV), virus morphology and replication. During the reported time period techniques in molecular biology have significantly advanced and were applied to IBDV allowing the implementation of reverse genetics to manipulate the virus *in vitro* and forwarded the understanding of virus characteristics. But also the understanding of the pathobiology and epidemiology of the virus progressed and the insights into the pathogenesis of the disease allowed further understanding of the mechanisms of immunosuppression and induction of immune reactions. Certainly, because of the major economic importance of the disease for the field, intervention strategies were improved and vaccine development pushed. Actually, many very effective vaccines have reached the market in these years and reduced field pressure if applied in an appropriate way. But challenges remain in many countries with vvIBDV and emerging novel antigenic variants. This is suggested to be due to interference of live IBDV vaccines with maternally derived antibodies (MDA) (18), and to incomplete antigenicity matches between vaccine and wild-type strains. Commercial vaccines may not induce sufficient protection against newly emerging strains (19,20,21).

## ETIOLOGY

**Classification: The discovery of serotypes, variants, and vvIBDV strains.** In the early 1980s, there were field observations of a respiratory syndrome in turkey poults, and the Saif laboratory was involved in research on the etiology of the disease. A bacterium, later identified as *Bordetella avium*, was recognized as the causative agent of the respiratory disease. During the search for an etiology, a virus later identified as serotype 2 IBDV, referred to as the MO virus, was isolated in Dr. Phil Lukert's laboratory. A similar virus, referred to as OH strain, was isolated in Mo Saif's laboratory. Serotype 2 IBDV was then finally identified by McFerran *et al.* in Northern Ireland in 1980 (22). Dr. Lukert was of the belief that this new serotype of IBDV was associated with the new respiratory syndrome in turkey poults. The National Turkey Federation (NTF) was seeking clarification of the etiology of the syndrome. The NTF asked Dr. Benjamin Pomeroy from the University of Minnesota, a prominent turkey health researcher, to convene a meeting with scientists involved in that research to seek a consensus. The meeting included John Barnes of Iowa State University at the time, Phil Lukert of the University of Georgia, Mo Saif of The Ohio State University, and Donald Simmons of North Carolina State University. The meeting did not resolve the issue, but heightened the need for more research on the new serotype of IBDV as to the possible role in the disease. During that period, three members of the Jackwood family (first Daral, followed by Deborah and Mark) were pursuing graduate work in the Saif laboratory. They all participated in different aspects of the IBDV research in chickens and turkeys with Daral most involved, and he continued working in that area until his retirement. These early studies illustrated that antibodies to both serotypes of the virus are widespread in commercial chicken and turkey flocks (23). More importantly, it was shown that serotype 2 viruses are infectious, but not pathogenic, in chickens and turkeys (24,25,26). Serotype 2 viruses replicated in the bursa of Fabricius (BF) but not in the lymphocytes. The virus was detected by direct immunofluorescence in the interstitial tissue surrounding the lymphocytes (Saif, unpubl. data). That also settled the controversy about the role of IBDV and

*B. avium* in the turkey respiratory syndrome, which was later designated Bordetellosis.

At that time in the early 1980s, when Mo Saif's team was working on these viruses, there were field reports from the Delmarva Peninsula of increased morbidity and mortality in commercial broiler flocks associated with respiratory signs. Dr. Gary Roundtree, a poultry veterinarian consultant at the Delmarva Peninsula, was aware of that work, and he was suspicious that a new IBDV type might be involved in that respiratory syndrome. He invited Saif in 1983 to visit some farms affected with the respiratory disease and to collect bursal samples for virus isolation. The Saif team detected IBDV designated as the MD isolate and studied the antigenic relationship of this isolate to viruses used in commercial vaccines at that time. These findings were reported at the condemnations meeting in 1984 (27). This new isolate (MD) was a serotype 1 virus, but cross virus neutralization studies indicated that it was antigenically significantly different from viruses used in commercial vaccines at the time. Later in 1987, Jackwood and Saif (28) conducted detailed studies that illustrated the antigenic diversity of IBDV. In 1991, Ismail and Saif (29) published a comprehensive study on the immunogenicity of the IBDV representing early isolates, vaccine strains, recent isolates including the variant viruses, and serotype 2 viruses, which are immunologically very different to serotype 1 viruses. The isolates recognized before 1983, designated classic viruses, protected against homologous viruses and provided partial protection against the variants, whereas the new variants provided full protection against homologous isolates and against the classic viruses.

Interestingly, Ismail et al. (30) isolated a virus (IN), with an antigenicity similar to the MD strain from an egg laying operation in Indiana that had IBD lesions and a drop in egg production, demonstrating that the new variant was not broiler specific and that these viruses were not confined to the Delmarva peninsula.

Meanwhile the respiratory problem was becoming widespread in the Delmarva area, causing serious economic losses. Dr. Thomas Holder, a poultry veterinarian working for a broiler company, invited several researchers working with respiratory diseases, including Saif, to a meeting in Salisbury, Maryland in 1984. Dr. Holder had assembled an impressive set of data from various flocks that exhibited the respiratory problem. Serological and isolation data for several poultry pathogens indicated an increase in antibody titers to IBDV. Otherwise, there were no other consistent patterns. Several presentations were given, including one about findings by the Saif team, and it became clear that further studies on IBDV should be pursued. Subsequently, Dr. Kenneth Eskelund from Maine Biologics Laboratory (MBL) asked Saif to provide the MD strain. One advantage of using that strain as a vaccine is that it had been adapted to cell culture. Dr. Eskelund made an inactivated vaccine using the MD isolate to vaccinate specific-pathogen-free (SPF) birds and challenged them with bursa homogenate tissues from affected flocks in the Delmarva area. He detected full protection in the vaccinated birds as compared to nonvaccinated contact controls. Dr. Eskelund was the owner of MBL and was acutely aware of the value of the vaccine and had enough data to prove it. MBL licensed the vaccine, which was highly effective in the field.

Meanwhile, Dr. Jack Rosenberger, who was present at Dr. Holder's meeting, isolated additional IBDV strains including A/Del, E/Del isolated from broiler flocks of the Delmarva peninsula, and GLS-5. These strains were further characterized using panels of monoclonal antibodies (31,32,33,34). Later it was shown that IBDV strains from the Delmarva region, isolated by the Rosenberger group, were antigenically and immunologically similar to the MD strain (35). Some of these isolates were also made into commercial vaccines using bursal homogenates as a source of the virus. Early on, it was claimed that Rosenberger's isolates could not be adapted to tissue culture, but the Saif lab was able to adapt all four strains. Another claim was that viruses harvested from bursa homogenates made better immunogens then cell culture–adapted viruses. Saif (unpubl. data) demonstrated that vaccines containing similar antigenic masses of tissue culture– and bursa-derived viruses elicited similar levels of virus-neutralizing (VN) antibodies and protection. Tsai and Saif (36) demonstrated in laboratory studies that tissue culture adaptation attenuated the pathogenicity of the viruses, but did not affect their antigenicity or immunogenicity. Over the years, claims of the emergence of new antigenic variants were made, but, unfortunately, some of these studies had major flaws, such as using unrealistic massive challenge doses. A standardized challenge dose for testing vaccine efficacy and pathogenicity studies of $10^2$ embryo infectious $dose_{50}$ ($EID_{50}$) was proposed by the WOAH (former OIE) and used by the U.S. Department of Agriculture Biologics to test for vaccine efficacy.

During that time in other parts of the world, highly virulent IBDV emerged. In 1988, the first outbreak with a highly virulent strain of IBDV was observed in East Anglia, England (37). This virus was subsequently further characterized in the context of a sequence analysis of some British isolates identifying genetic drift leading to a difference of at least 29 bases within the virus protein (VP) 2 region compared to the classical strains (38,39). Although amino acid changes in VP2 were used to differentiate this highly virulent IBDV from classic strains, together with a panel of neutralizing monoclonal antibodies in the antigen-capture ELISA, these criteria were shown not to be sufficient for the characterization of IBDV pathotypes. *In vivo* studies were suggested to complement the characterization (40). VP2 was shown more than 10 yr later not to be the sole determinant of the very virulent nature of this virus (41). VP1, the RNA-dependent RNA polymerase protein, contributes also to the increased virulence of the virus through more efficient virus replication, causing lesion development in thymus and spleen (42). Phylogenetic analysis indicated that VP1 of vvIBDV is phylogenetically distinct from other IBDV classical virulent/attenuated or serotype 2 strains, suggesting a different origin and segment reassortment (43,44).

These highly virulent strains did not grow in tissue culture and were designated as vvIBDV because of their pathogenicity (45). vvIBDV spread quickly to other parts of the world, including Europe (37), Asia (20), and South America (46), and were also reported by Professor Müller's group in Africa (47), replacing the classical strains in these parts of the world. It was not until 2008 that vvIBDV strains appeared also in the United States (48).

Outbreaks with vvIBDV were characterized by a sudden onset of depression and high mortality of as much as 60%, in not only young but also older birds, despite vaccination (39,49,50,51,52). Lesions include not only severe lymphoid cell depletion in the bursa but also in other nonbursa lymphoid organs coinciding with virus replication, and hemorrhages in the muscles and proventriculus, which are not observed for milder classical or antigenic variant strains (50). Differences in pathogenesis and immune response, especially cytokine mediated, may account for the acute vv pathotype, and the fast onset of high mortality rates in unprotected flocks (50). Antigenically vvIBDVs are similar to the classic IBDV strains, and vvIBDV-induced mortality may be controlled by partially attenuated classic IBDV vaccine strains (52,53). But mutations such as at position 222 of VP2 from Proline in classic IBDV to Alanine in vvIBDV may allow slight changes in the conformation of

epitopes, which are sufficient to escape neutralization by antibodies induced by classical strains (49). Phylogenetic analysis of vvIBDV strains from various countries suggest a common ancestor, and subsequent independent evolution of vvIBDV in different regions (50). The neutralizing epitopes are localized in the variable domain of VP2 being highly hydrophobic and flanked by two major hydrophilic peaks (49). Therefore, new vaccination concepts needed to be developed to control this economically important pathogen, including less attenuated, more invasive live strains breaking early through interfering maternally derived antibody levels (50). Further details will be addressed in the next history episode of IBDV.

The basis for safety investigations of IBD vaccines was set during that time by Guittet et al. proposing parameters to be investigated such as bursal damage and assessment of immunosuppression measured by titers to Newcastle disease (ND) vaccination (54).

**Morphology and virus replication.** With classical virology approaches and the advances in molecular biology, the characterization of IBDV progressed at the end of the 1970s. The double-stranded nature of the genome, as well as the presence of two genome segments, was established by a German working group in Giessen (55), which coincided with further biophysical and biochemical characterization of bisegmented dsRNA viruses (56). These findings suggested that a new taxonomic classification was needed. The early discoveries on the structural characteristics were summarized by Becht (57). In the following years viral genes and proteins were further characterized, IBDV was classified as a member of the *Birnaviridae*, and the processing of virus polyprotein and replication characteristics were described (58,59,60,61). These developments also allowed the identification of the structural peptide responsible for the induction of VN antibodies and subsequent protection (62), and the description of the three-dimensional structure of the IBDV virions, including the trimeric subunits of the capsid (63). Müller and his team in Giessen conducted these studies on IBDV structure and biological characteristic as part of his habilitation thesis in 1986 (64). Further studies revealed a variable region within the VP2 as the target for VN antibody binding (65,66), and the genetic basis for antigenicity was discovered (67). These findings set the basis for the development of new generation recombinant as well as subunit vaccines against IBDV (68,69,70). The other structural polypeptide VP3 was shown by Oppling *et al.* (71) in 1991 to carry group- and serotype-specific epitopes. The nonstructural protein VP5 was identified by the same working group some years later, and subsequently demonstrated to be involved in the control of virus release (72).

**Reverse genetics as the basis for understanding virus characteristics.** At the beginning of the 1990s, Vakharia and co-workers used PCR for efficient cloning of IBDV segments (73). Additionally, Mundt advanced IBDV research by describing the full length sequence of both genome segments of IBDV serotype 1 and serotype 2, while he was residing at Hermann Müller's lab in Germany (74). This paved the way for further joint work between Mundt and Vakharia. When Egbert Mundt spent some time as a visiting scientist with Vakharia at the University of Maryland, USA, in the mid-1990s, they discovered that synthetic transcripts of IBDV are infectious and the first reverse genetics system for a double-stranded RNA virus was developed (75). The system was modified by different groups and used in the following years to mutate IBDV to understand more about virulence determinants and pathogenesis of the disease (76,77,78,79).

## EPIDEMIOLOGY AND PATHOBIOLOGY

**Wild birds.** Studies from the 1990s confirmed serologically the circulation of serotype 1 as well as serotype 2 strains in sedentary as well migratory birds (80). Anti-IBDV antibodies were detected in ostriches (81), adelie and emperor penguins (*Pygoscelis adeliae* and *Aptenodytes forsteri*, respectively) in the Antarctic (82), common eider (*Somateria mollissima*) females and immature herring gulls (*Larus argentatus*) in the Baltic Sea, and in blood of spectacled eider (*Somateria fischeri*) females nesting in a remote area of western Alaska (83) as well as various raptor and passerine species (80). Also the detection of IBDV in the lesser mealworm fed with IBDV-contaminated feed suggests it as a possible vector (84).

**Genetic basis for susceptibility in chickens.** Bumstead *et al.* demonstrated a genetic component in the susceptibility of chickens for IBDV (85), which was also confirmed by other working groups in the following years. An impact of the major histocompatibility complex (MHC) haplotype was suggested, but the mechanisms behind the variation in virus control and immune response could not fully be elucidated (17,86,87,88).

## PATHOGENESIS OF THE INFECTIOUS PROCESS

**Immunity and immunopathogenesis.** Various attempts were undertaken to identify the IBDV cellular receptor using cell culture systems such as chicken embryo fibroblast, and lymphoid cells from various tissues (89,90) but still today, this mystery is not fully solved (90). Since the original observation by Faragher and associates (91,92) that IBDV infection decreased antibody responses to immunization with vaccines and protection against challenge with NDV, most of the research on immunosuppression between 1975 and 1995 has been descriptive rather than mechanistic. It was noticed early by Winterfield et al. (93) that infection with IBDV caused severe atrophy of the BF, which can last up to 71 days postinfection (dpi). Up to 51 dpi, the bursal follicles were devoid of lymphocytes with repopulation noticed at 71 dpi. The effect of IBDV infection on peripheral blood lymphocytes (PBL) was examined originally using anti–B and anti–T cell antisera (94,95), showing a decline of circulating B cells up to 8 wk postinoculation, but no effect on circulating T cells. Rodenberg et al. (96) confirmed and extended these findings using flow cytometric analysis and monoclonal antibodies for IgM+ B cells and CD4+ and CD8+ T cells. Their results showed a significant decrease in IgM+ B cells in the spleen and BF. One of the major mechanisms by which IBDV causes lesions in a strain-dependent way is apoptosis, which was shown by various working groups in the 1990s (97,98,99,100). Not only IBDV-positive but also virus-negative cells may show apoptosis because of bystander effects or virus-associated impairment of the withdrawal of apoptotic cells (99,101). Overall, it was suggested that IBDV-induced apoptosis is a multistep process involving IBDV replication, protein expression, and virion release (102), but the viral proteins and pathways important for the induction of the programmed cell death were not fully elucidated at that time (76,103,104).

The relative proportions of CD4+ and CD8+ T cells in the spleen and PBL were not changed after IBDV infection (96). Infection of 1- and 21-day-old chickens caused a temporary reduction of plasma cells in the Harderian gland (HG) (105,106). Antibody responses in the HG against *Brucella abortus* antigen and sheep red blood cells (SRBC) were significantly decreased in parallel with the

decreases in serum antibodies, although the responses recovered over time (107).

After the original observation that IBDV infection in young chickens impaired antibody responses to NDV (91,92), many researchers confirmed these findings against NDV, IBV, and infectious laryngotracheitis virus, *Mycoplasma synoviae*, and *Eimeria tenella* (107,108,109,110,111). Unfortunately, with very few exceptions (e.g., Nakamura *et al.* [112]), these experiments were done with IBDV isolates without knowledge on the presence or absence of CIAV.

The effect of IBDV infection on MD has also been investigated, but the results were not convincing that IBDV enhances MD or interferes with vaccine-induced protection. Cho (113) and Giambrone *et al.* (114) reported that placing chickens into an IBDV-contaminated environment increased the incidence of MD in both herpesvirus of turkeys (HVT) and vaccinated and nonvaccinated chickens. Vaccination with HVT in IBDV-infected birds resulted in significantly lower levels of HVT-specific antibodies compared to the antibody levels in absence of IBDV infection (115). Because vaccine-induced immunity to MD is mostly based on cytotoxic T lymphocytes (CTL) and natural killer (NK) cells and not on the presence of antibodies, the interpretation of these results is difficult. We may speculate that in the study by Giambrone *et al.* (114) birds may have been exposed to CIAV in addition to IBDV, which was not uncommon in many experimental studies in the time before the identification of CIAV. Infection with CIAV interferes with the development of antigen-specific CTL and IgY and IgA antibodies because of the decrease in T helper cells (116,117). Inoculation of 1-day-old chicks with HVT and IBDV reduced the protection against challenge with MDV at 6 days of age, but when challenged at 14 days of age protection was not affected (118). The lack of protection after early challenge may be the result of interferon induction by IBDV (119) causing a delayed replication of HVT. Interestingly, combined infection of IBDV and MDV at 2 days of age (120) or IBDV followed by MD challenge at 6 days of age (118) caused a decrease in MD incidence, probably because the early replication of MDV may start in B lymphocytes (121), which are reduced by IBDV infection. IBDV infection of chickens latently infected with MDV did not result in reactivation of the lytic MDV infection (122).

The lack of antibody responses to vaccination with ND vaccines led Ivanyi and Morris (123) to examine the effects of infection at 1 versus 21 days of age on the antibody classes. Infection with IBDV at 1 day of age resulted in decreased humoral responses to IBDV, human serum albumin, and SRBC. The IgM response consisted of the 7S monomeric form with the disappearance of IgM with the allotypic marker $M1^a$, suggesting that B lymphocytes surviving IBDV infection were altered and slowly replacing B cells seeded prior to infection.

Early studies had suggested that cell-mediated immune (CMI) responses were not impacted by IBDV infection based on skin allograft responses (124), but Panigrahy *et al.* (125) found that IBDV infection prolonged the survival time of the allografts. Sharma and Lee (126) using mitogen stimulation of spleen lymphocytes with phytohemagglutinin found a reduced response within the first 2 wk postinfection confirming data reported by Confer et al. (127). Long-lasting depressed mitogen responses have also been reported (128). The short-term depressed response to mitogen stimulation was not due to a direct effect on the T cells, but was caused by "suppressor macrophages" (126). The effect of infection on NK cells was not consistent, and depended on individual birds: some birds had an increased response, whereas other birds in the same experiment showed a depressed response (126). Jagdev M. Sharma, who was appointed as Professor and Pomeroy Endowed Chair in Avian Health at University of Minnesota in 1988, together with his team made significant contributions to the understanding of the CMI in IBDV. As more tools to investigate the chicken cell-mediated immune response came available his research focused on the involvement of T cells in IBDV pathogenesis, demonstrating their role in early control of local virus replication (129,130,131,132). On the other hand, T cell-intact birds showed in comparison to Cyclosporin A treated and thymectomized chickens a delayed recovery from IBDV-induced lesion development (132). Subsequently his group focused on the involvement of macrophages in IBDV pathogenesis using *in vitro* and *in vivo* models (133), and he suggested that they were target cells for IBDV (133).

In the mid 1990s, the first *in vitro* and *in vivo* studies on the effects of IBDV infection on cytokine responses were reported, but the majority of the studies on cytokines were reported after the period covered in this review (134,135).

## DIAGNOSIS

Traditional virological methods were used during the early days to isolate IBDV in embryonated eggs and later in various cell culture systems. This was followed by the development of molecular tools such as PCR in the early 1990s by Dr. C. C. Wu *et al.* at the Mississippi State University, USA (136,137). In a follow-up study primer pairs were designed based on the hypervariable region of VP2 to allow differentiation of serotypes 1 and 2 (138). Dr. Wu continued her research in this field when she moved to Purdue University. The application of DNA sequencing techniques in the mid 1990s by various groups in the United States, Europe, and Asia allowed epidemiological investigations and the identification of specific amino acid mutations in VP2 and VP1, which are associated with classical, very virulent, and antigenic variants (38,139,140,141,142,143,144), which led later in 2018 and 2021 to a genotypic classification of the virus (145,146). By the end of the 1980s and 2000 at the Ohio State University, Daral Jackwood and Saif and their teams advanced our knowledge in the molecular detection and differentiation of IBDV significantly (28,140,147,148,149). These techniques are used today for epidemiological investigations and may allow also retrospective analysis of newly emerging strains and the contribution of reassortment and/or recombination events to these viruses, as demonstrated for example for vvIBDV strains in different regions in the world (150).

Although the agar gel precipitation test was used in the early days, new serological techniques were also further developed. Indirect ELISA and other systems such as ELISPOT assays became commonly used starting in the 1980s (151,152,153). The ELISA is currently the most used serological test in the field, for example, allowing the detection and quantification of MDA at the flock level. This led to the discovery that MDA may interfere with the IBDV-vaccine response (154,155). The detection of MDA interference with vaccination led to the establishment of the "Deventer formula" in the Netherlands to estimate the right time point for vaccination (156). This method is widely applied throughout the world to vaccinate progeny, especially in the face of a high IBDV field pressure.

VN antibody tests were also used in the indicated time period and continue to be important not only for research, but also for vaccine development to determine possible cross-reactivity (157,158). During the COST action 839 the first European ring test for IBD serological diagnosis was organized with 16 participating laboratories throughout Europe. The VN test was confirmed as the most sensitive test, and ELISAs may show the best correlation with it compared to the traditional agar gel precipitation test (159). The VN test allows the differentiation between serotypes, while the ELISA may not differentiate antibodies against serotypes 1 and 2 IBDV strains (35). Overall, significant variations in the context of MDA quantification between laboratories can be noted (160,161), and therefore, the working group of Kreider recommended the introduction of reference sentinel sera.

## INTERVENTION

**Discovering the structure of IBDV followed by the generation of new-generation vaccines.** With the development of the *in ovo* vaccination technology by Sharma and Burmester in 1984 (162) the ground was set to a new delivery route for various vaccine formulations (163) including new-generation vaccines such as IBDV immune complex vaccines (164). Immune complex vaccines were first described in 1997 by Haddad *et al.* in the United States (165), and have reached the market later on. They are used commonly in South America and Europe but not used or used less often in other regions, including the United States. Also, the first HVT-IBDV-recombinant vaccine candidate was developed by the industry in France (166). Currently, various HVT-IBDV vaccines have reached the market. Other vectored IBDV-vaccine candidates were experimentally evaluated (69,167,168) but not all have a market share currently. DNA vaccines have been tested experimentally, but were shown to be less protective than other vaccine types (169), and therefore have not been licensed so far. Subunit vaccines were also investigated using baculovirus-expressed virus-like particles, or different viral proteins, with variable success of protection (170,171).

Most of the live IBDV vaccine strains can cause some damage in the BF (172,173) and, depending on the type of vaccine strain, this may occur in birds when maternal antibody levels are decreasing. The damage, which is in principle similar to the immunosuppressive effects described previously, is generally outweighed by the benefits provided by the protection against clinical disease. Although various live vaccines have reached the market during the reported period, the control of vvIBDV has been especially challenging. The development of live intermediate plus vaccines allowed to break through interfering maternally derived antibodies early, and therefore induce protection against high vvIBDV field pressure. But still today, field outbreaks of vvIBDV may occur, and continue to be a challenge in the field (18,19,20,21). The review "Current status of IBD vaccines" by Müller *et al.* (174) summarized and elaborated many aspects of IBDV vaccination, and further references can be found there.

Overall, the vaccination strategy may vary between countries and regions in the world; some rely on the transfer of high MDA levels to progeny through efficient hen vaccination, and others consider an early IBDV-vaccination time point of progeny more desirable. The use of autogenous vaccines may complement the licensed vaccines by allowing to protect against constantly evolving antigenic variants, especially if geographic clusters occur with a limited market (45).

## CONCLUSIONS

Between 1977 and 2005 many IBDV working groups were formed all over world, and it was an important time for IBD research. Major advancements were achieved with respect to identifying variant strains and serotype 2 IBDV and the appearance of vv strains during the late 1990s. The bisegmented double-stranded nature of the IBDV RNA genome was recognized, which led to the subsequent classification of IBDV as a birnavirus. Characterization and elucidation of the viral proteins led to the understanding of the importance of VP2 as the major protein responsible for the attachment of the virus to cell receptors, which remained elusive during the period reviewed in this article. The development of monoclonal antibodies to identify B and T lymphocytes as well as macrophages led to a better understanding of the impact of IBDV on IgM+ B cells, while T cells are not directly affected. Macrophages and T cells were shown to be important for the response to IBDV infection in the BF. The use of reverse genetics allowed the construction of mutant viruses, which was also an important development to study the pathogenesis of infection. New vaccine approaches such as antigen–antibody complex vaccines and especially the use of recombinant HVT vaccines expressing IBDV VP2 opened avenues to improve vaccine efficacy in the face of MDA. The development of ELISA kits for the detection of IBDV antibodies facilitated the monitoring of vaccine efficacy as well as epidemiologic studies. Large sequence data sets of circulating IBDV strain sequences from around the world became available, leading to a better understanding of virus evolution and suggestions for new IBDV nomenclature (145,146). The first historical review of IBD (2) ended with the suggestions that the next 20 yr would show major progress, which they certainly did, and we know by today that the period after 2005 also provides major advances, especially in the understanding of the molecular pathogenesis at the cellular level. Because of the implementation of very effective vaccination strategies, the clinical disease is well controlled in many regions these days, but immunosuppression may still occur and contribute to health problems in birds and to economic losses. Therefore, proactive surveillance is important to adjust current vaccines and prophylactic strategies to newly emerging IBDV strains and bird genotypes on an ongoing basis.

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